

Determination of carotene, tocopherols and tocotrienols in residue oil from palm pressed fiber using pressurized liquid extraction-normal phase liquid chromatography

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Abstract

Pressurized liquid extraction (PLE) along with normal phase liquid chromatography (NPLC) is evaluated as a new approach for the determination of β -carotene and Vitamin E isomers in residue oil obtained from palm pressed fiber (PPF). The relative extraction recoveries obtained by optimized PLE were found to be equivalent ($\sim 100\%$) to conventional Soxhlet extraction. Optimized PLE was obtained (5 g of sample, 80°C , 1500 psi, 2×10 min static extractions with flush volume 50%) combined with NPLC system. Typical extractions give $3.7\text{--}4.0\text{ mg ml}^{-1}$ of Vitamin E isomers and β -carotene of $3.3\text{--}3.5\text{ mg ml}^{-1}$, which is similar to or greater than classical Soxhlet extraction with *n*-hexane or chloroform. The new developed method demonstrated an acceptable performance with good efficiency in terms of total extraction time, total solvent usage, total β -carotene and Vitamin E isomers contents as well as exceptional method repeatability.

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Keywords: Pressurized liquid extraction; Liquid chromatography; Tocopherol; Tocotrienol; Palm oil

1. Introduction

Tocopherol was first reported in 1922 by a biologist, Evans when they studied the reproduction of the rat [1]. In 1937, Emerson et al. [2] described the existence of various Vitamin E homologues having different abilities to prevent Vitamin E deficiency. The naturally occurring tocopherols and tocotrienols constitute the majority of the Vitamin E group of compounds and they are composed of eight vitamers: α -, β -, γ -, and δ -tocopherol and their four corresponding unsaturated congeners, namely α -, β -, γ -, and δ -tocotrienol [3]. Tocopherols are methyl-substituted hydroxychromans with a saturated phytyl side chain and the tocotrienols with an unsaturated side chain (Fig. 1). The main interest for studying Vitamin E is its natural antioxidant capabilities. It has been known as the most efficient antioxidant for breaking free radical chain reactions and provides some natural

oxidative protection to the oil. Most vegetable oils and biological fluids contain varying amounts of the dominant tocopherols. Nevertheless, tocotrienols can be found in palm oil, coconut oil, and cereal grains such as wheat, rye, oats, and barley [4].

Crude palm oil (CPO) is one of the major sources of Vitamin E and contains high quantities of tocopherols and tocotrienols in the range of 600–1000 ppm [5]. Recently, palm fatty acid distillate (PFAD), a by-product of the physical refining of palm oil, has been identified as a good raw material source for the recovery of Vitamin E. PFAD is relatively cheap and readily available throughout the refining industry [6].

Palm pressed fiber (PPF) is the fibrous residues separated from the mesocarp and kernel during palm oil extraction. PPF is usually burned as fuel to provide energy for the processing mill. Oil losses usually occur in various by-products, including the fiber, which remains after the mesocarp oil is extracted by a screw press. Because the screw press process for the extraction of oil from mesocarp is conventionally

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done by single stage pressing, the oil loss is considerable [7].

Residue oil obtained from the PPF has been known as a good source of Vitamin E from the middle of 1990s [7]. Classical Soxhlet extraction had been utilized as the extraction method to obtain the residue oil from the PPF. Although the residue oil contains high quantities of minor components including the Vitamin E isomers, high quantities of impurities and degradation product were observed in the analysis of the oil by various analytical methods.

Pressurized liquid extraction (PLE) is a new extraction procedure that combines elevated temperature and high pressures with liquid solvents to achieve rapid and efficient extraction of analytes from various matrices. PLE has recently gained wide acceptance for the analysis of different pesticides, PAHs, PCBs in sediments and sludge [8–10]. Moreover, PLE has been shown to be equivalent to standard EPA extraction methodology in terms of recovery and precision [11]. However, no reports of the work performed to extract residue oil in PPF have been published to date.

In the present study, the optimization and validation of pressurized liquid extraction (PLE) was investigated as a new approach for the rapid extraction of residue oil in PPF. PLE method is compared with the Soxhlet extraction in determining the valuable tocopherols and tocotrienols components in the recovered oil obtained from PPF. In addition, optimization of Vitamin E isomers separations using NPLC at different column temperature is also described and subsequently utilized as a separation technique in analyzing the Vitamin E contents in residue oil.

2. Experimental

2.1. Reagents

Tocopherols and tocotrienols were purchased as isomer kits from Merck (Darmstadt, Germany). α -Tocopherol acetate (α -TAc) was obtained from Sigma–Aldrich (USA). Chloroform, *n*-hexane, 2-propanol, diethyl ether, ethyl acetate, absolute ethyl alcohol, and 1,4-dioxane were analytical grade obtained from various suppliers.

2.2. Chromatographic conditions

The LC systems consisted of a conventional LC system coupled with a column oven of a Perkin Elmer Autosystem Gas Chromatography (USA). NPLC separations were carried out on a 5 μ m Hypersil silica column (200 mm \times 4.6 mm i.d.) (Sigma–Aldrich, Milwaukee, USA) using a Waters 515 HPLC pump (Milford, USA) for mobile phase delivery. A Rheodyne 7125 injection valve (Cotati, USA) fitted with a 5 μ l loop was used for sample introduction. Analyte peaks were detected using a Shimadzu SPD-6A UV detector (Kyoto, Japan) and were recorded on a Hewlett Packard HP 3396 Series II integrator (USA).

2.3. Separation of tocopherols and tocotrienols by NPLC at different temperatures

Samples of eight natural Vitamin E isomers (α -tocopherol, β -tocopherol, γ -tocopherol, δ -tocopherol, α -tocotrienol, β -tocotrienol, γ -tocotrienol, and δ -tocotrienol) and α -tocopherol acetate were dissolved in the *n*-hexane and stored

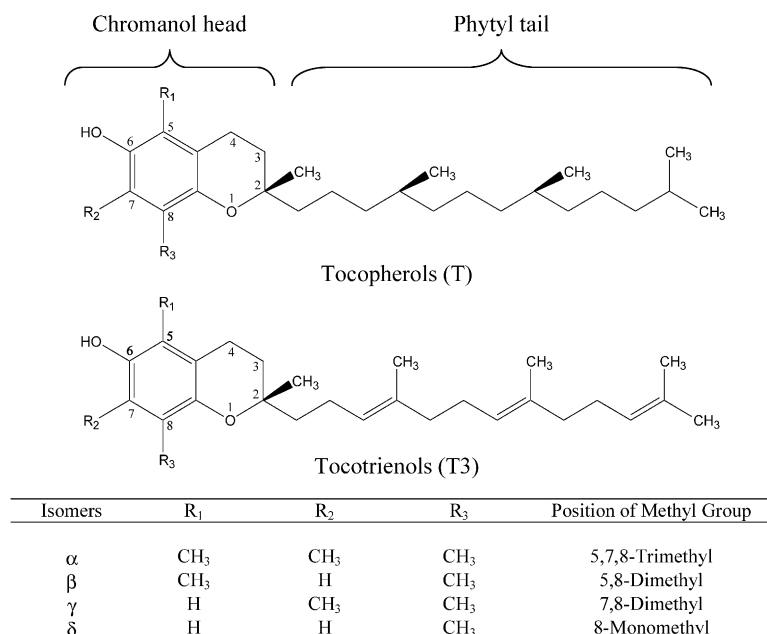


Fig. 1. Chemical structures of tocopherols and tocotrienols.

at temperature -18°C in darkness. Separation of prepared mixture was carried on a Hypersil silica column using mobile phase *n*-hexane:1,4-dioxane (96.0:4.0 v/v) at different column temperatures (30 – 60°C). The prepared mixture was injected in triplicate onto the column and the solute concentrations were 0.1 – 0.3 mg ml^{-1} . UV detection of analytes for the comparison study was at 295 nm .

2.4. Palm pressed fiber samples

PPF samples were obtained from Felda Palm Industries Sdn. Bhd. at Kota Tinggi, Johor, Malaysia. Freshly collected samples were dried at 60 – 70°C for 1 h. Homogenization was performed with a grinder.

2.5. Extraction of residue oil from palm pressed fiber

2.5.1. Soxhlet extraction

Separate extractions of dried fiber (5 g) were performed with different solvents, namely *n*-hexane and chloroform in a Soxhlet apparatus for 8 h. The residue oil obtained was weighed after rotary evaporation to remove solvents and pump-dried under vacuum.

2.5.2. Pressurized liquid extraction

Pressurized liquid extraction of residue oil from PPF samples was performed using a home-made accelerated solvent extractor (Fig. 2). Dried fiber samples (2, 4, 5, and 6 g) were accurately weighed into a 32 ml high-pressure extraction vessel. The vessel full of fiber was placed into the heated compartment with its inlet and outlet connected to the preheating coil and the static valve C2, respectively. Extractions were carried out using 100% *n*-hexane. The extraction solvent was pumped through the vessel with static valve C2 open and the valve was closed once the solvent started to exit. Static valve C1 was closed after the system reached the expected pressure and subsequently the vessel was heated. Temperatures of 60, 80, 100, and 120°C and pressures of 1000, 1500,

2000, and 2500 psi were tested with a heat-up time of 5 min. One-cycle extractions were performed for 10 min in static mode with flush volume 50% of the extraction cell volume (the volume of fresh *n*-hexane used for flushing of the extraction cell after first 10 min static extraction, i.e. approximately 16 ml, was collected with static valves C1 and C2 opened). Another 10 min static extraction was then carried out using same operating condition. The extracts were cooled to 40°C by reducing oven temperature and were subsequently purged from the vessel using pressurized nitrogen for 1 min. The total volume of extract collected was in the range of 45–48 ml. The residue oil obtained was weighed after rotary evaporation to remove solvents and pump-dried under vacuum.

2.6. Determination of tocopherols, tocotrienols and β -carotene content

Tocopherols and tocotrienols contents were determined by NPLC. Residue oils obtained from Soxhlet and PLE extractions were pretreated according to the method described by Tan and Brzuskiwicz [12] by dissolving the residue oil in *n*-hexane and chill overnight at -20°C , respectively. Samples were then centrifuged at 4°C to remove solids, and the lipid soluble supernatant was chromatographed. A solvent system of 99:4 v/v (*n*-hexane:1,4-dioxane) with a flow rate of 1 ml min^{-1} was applied to the system, and all the analytes were detected using UV detector set at a wavelength of 295 nm. Identification of analyte was carried out by comparison of retention times in chromatogram with that of tocopherols and tocotrienols standards.

The carotene content of residue oil is defined and calculated as β -carotene in mg ml^{-1} by using PORIM test method p2.6 (1995) [13]. Residue oil obtained (0.1 g) was weighed into the 25 ml volumetric flask. The test portion was dissolved with *n*-hexane and diluted to the mark. The test portion was measured using a UV–vis detector with absorbance at 446 nm against the solvent used.

3. Results and discussion

3.1. Separation of tocopherols and tocotrienols by NPLC at elevated temperature

Separations of tocopherols and tocotrienols were carried out at different column temperatures from 30, 40, 50 and 60°C . It was noted that the elution times were inversely proportional to the column temperature. Based on the retention factors obtained in our study, it was observed that for each isomer, the retention factor decreased by a factor of 1.08 when the column temperature was increased by 10°C . This observed trend was found consistently for the Vitamin E isomers at all column temperatures studied.

The results also showed that the column efficiency was directly proportional to column temperature. This was probably due to the increase in the diffusion coefficients of the mobile

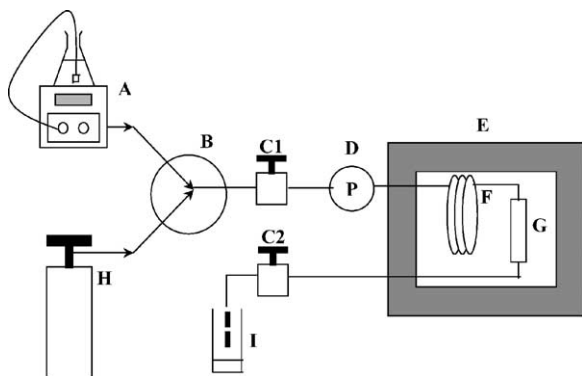


Fig. 2. Schematic diagram of home-made pressurized liquid extraction (PLE) system: A, HPLC pump; B, three-way switching valve; C1 and C2, static valves; D, pressure gauge; E, oven; F, preheating coil; G, extraction vessel; H, pressurized nitrogen gas; I, collection vial.

phase and the analytes with the increase in column temperature. The resolution data gathered in our study demonstrated that the best separation was achieved at a column temperature of 40 °C. The repeatability and reproducibility of the method developed were also investigated. It was found that the results achieved on the same working day were generally acceptable, with R.S.D. value of <5%, and on three consecutive days, the R.S.D. values were lower than 7%. The detection limit values, expressed as amount injected, were 5 ng (δ -tocopherol and δ -tocotrienol), 10 ng (γ -tocopherol, β -tocopherol and β -tocotrienol), 15 ng (α -tocopherol), and 20 ng (α -tocotrienol and γ -tocotrienol).

3.2. Determination of Vitamin E contents in residue oil from palm pressed fiber

3.2.1. Soxhlet extraction technique

Two types of solvent were used: *n*-hexane and chloroform. The PPF sample amount and total solvent usage were optimized based on the volume of the extraction thimble and size of the Soxhlet apparatus. Eight hours of Soxhlet extraction is generally adequate for exhaustive extraction of the residue oil in PPF sample. Hence, by using Soxhlet extraction as the reference method, the PLE method can be easily optimized based on the weight of the residue oil extracted from the PPF. It can be observed that the major forms of Vitamin E isomers present in residue oil from PPF using *n*-hexane as extraction solvent are α -tocopherol (55.2%), followed by γ -tocotrienol (24.9%), α -tocotrienol (17.2%), and δ -tocotrienol (2.8%). On the other hand, the major forms of Vitamin E isomers present in residue oil from PPF using chloroform as extraction solvent were α -tocopherol (61.6%), followed by α -tocotrienol (19.6%), γ -tocotrienol (18.6%), and δ -tocotrienol (0.2%). There was no significant deviation in the total quantities of tocopherols and tocotrienols obtained in PPF in relative with the results obtained by a previous study [7]. *n*-Hexane has been proven as a more suitable organic solvent to be utilized as the extraction solvent.

3.2.2. Pressurized liquid extraction technique

Recoveries obtained by Soxhlet for the residue oil from PPF were compared with recoveries obtained by PLE in order to obtain optimal experimental conditions.

3.2.2.1. Choice of the sample amount. The optimization of sample amount for extraction is important to achieve the best phase equilibration between sample matrices and extraction solvent in extraction vessel. In addition, the sample amount filled into the vessel will significantly reduce the total solvent usage for each extraction. In this work, optimization was performed on 2, 4, 5, and 6 g samples with a temperature of 100 °C, pressure of 1500 psi, and *n*-hexane as extraction solvent using the same extraction vessel. Fig. 3(a) showed the result obtained in the form of relative extraction recoveries (for Soxhlet recoveries considered as equal to 100%). It can be observed that under these experimental conditions,

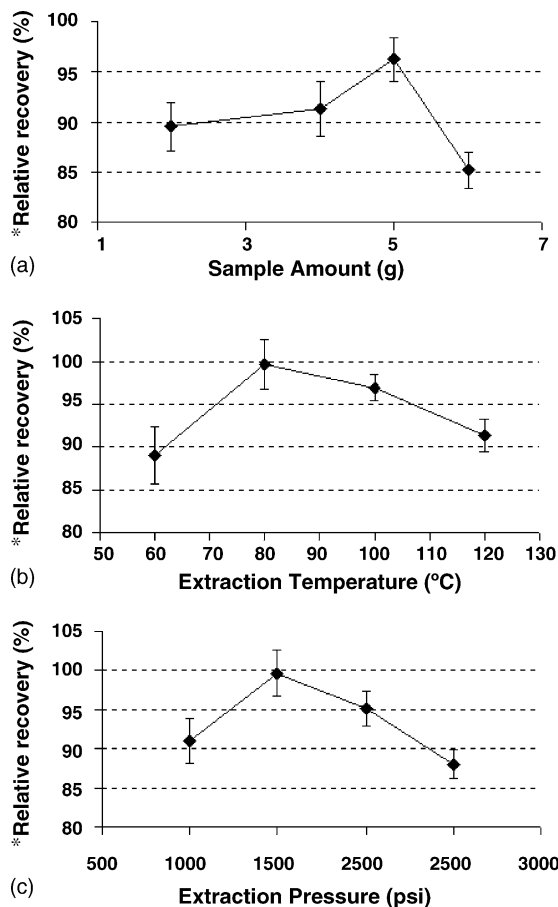


Fig. 3. Effect of (a) sample amount, (b) temperature, and (c) pressure on pressurized liquid extraction (PLE) efficiency (error bars represent standard deviation of results, $n = 4$); * (weight of residue oil obtained using PLE/weight of residue oil obtained using Soxhlet) $\times 100$.

recovery was highest for the 5 g sample. However, a decrease of recovery was noted for the 6 g sample. Therefore, 5 g of sample amount was chosen as the optimum sample amount for PLE in this study.

3.2.2.2. Choice of the extraction temperature. Temperature is the most important parameter used in PLE extraction. When the temperature is increased, the viscosity of the solvent is reduced, thereby increasing its ability to wet the matrix and solubilize the target analytes. High temperature also assists in breaking analyte–matrix bonds and encourages analyte diffusion to the matrix surface [14]. Extractions were performed on 5 g samples at 60, 80, 100, and 120 °C and a pressure of 1500 psi with *n*-hexane as the solvent. Relative extraction recoveries (Soxhlet recoveries considered as equal to 100%) are represented in Fig. 3(b). At 60 °C the extraction generally resulted in poor relative recoveries. This might be due to the extraction solvent that was still below the superheated state (boiling point of *n*-hexane: ~ 69 °C) that led to a decrease of solvent penetration strength. An increase of extraction temperature to 80 °C dramatically increased the relative recoveries to the values comparable with the Soxhlet extraction

(~100%). An increase of temperature to achieve its superheated liquid state (above solvent boiling point) generally resulted in better mass transfer rates between the solvent and the sample matrices and directly increases the capacity of solvent to solubilize analytes. When the temperature was increased to 100 and 120 °C, no further increase of relative recoveries was observed. In contrast, slightly decreased recoveries were noted. This phenomenon could be due to the vaporising of the hot solvent that led to a decrease in recovery. However, this phenomenon was proven not to give a marked effect on the actual extraction efficiency due to the low heat capacity of the organic solvent and rapid loss of heat along the adequately lengthy tubing between extraction vessel and collection vial (~30 cm) [15]. Based on the results gathered, extraction temperature of 80 °C was chosen as the optimum temperature for the extractions in this study.

3.2.2.3. Choice of the extraction pressure. The main purpose of high pressure in PLE is to maintain the solvents as liquids while above their atmospheric boiling points. In this study, extractions were performed on samples (5 g) under different extraction pressures of 1000, 1500, 2000, and 2500 psi at a temperature of 80 °C with *n*-hexane as the solvent. Relative extraction recoveries (Soxhlet recoveries considered as equal to 100%) are represented in Fig. 3(c). Based on the results obtained, recoveries are increased radically from 1000 to 1500 psi. The use of appropriate pressure will facilitate extractions from samples in which the analytes have been trapped in matrix pores. The pressure forces the solvent into areas of the matrices that would not normally be contacted by solvents under atmospheric conditions [15]. However, recoveries were gradually decreased when higher pressures (2000 and 2500 psi) were applied onto the system. Based on the results on the relative recoveries obtained in this study, a pressure of 1500 psi was chosen as the optimum pressure for the extraction.

3.2.2.4. Quantitation of tocopherols and tocotrienols using optimized PLE method. Based on the results obtained from the optimization of PLE on PPF, the following optimum extraction conditions were chosen: sample amount, 5 g; extraction temperature, 80 °C; extraction pressure, 1500 psi with a heat-up time of 5 min, one-cycle of extractions were performed during 10 min in static mode with 50% flush volume of extraction solvent. The extracts were purged from the extraction vessel using pressurized nitrogen for 1 min. Fig. 4 shows the separation of PLE extraction yields of tocopherols and tocotrienols in residue oil obtained from PPF. It can be observed that the major forms of Vitamin E isomers present in residue oil from PPF are α -tocopherol (52.5%), followed by γ -tocotrienol (28.2%), α -tocotrienol (17.4%), and δ -tocotrienol (1.9%). The overall results showed that residue oil contain high quantities of Vitamin E isomers in a range from 3.7 to 4.0 mg ml⁻¹, which is comparable or even higher than the results obtained using Soxhlet extraction.

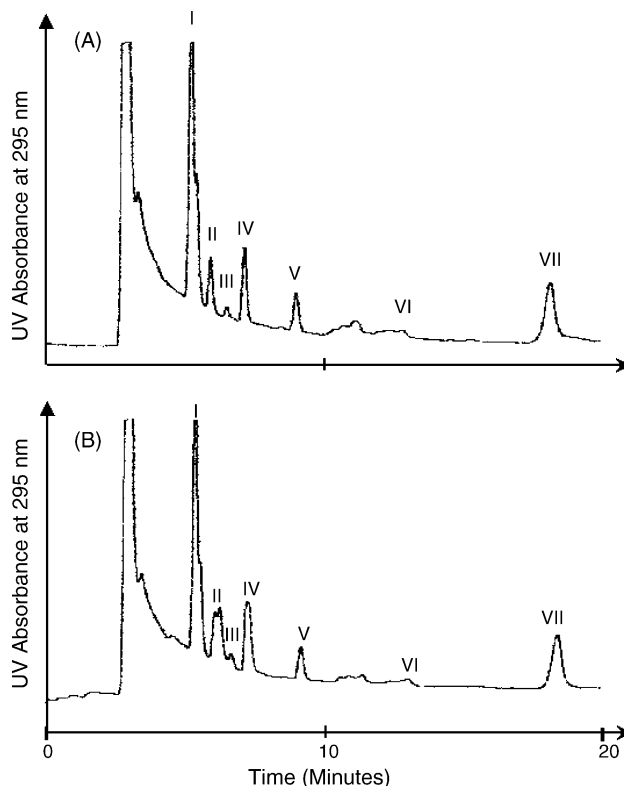


Fig. 4. NPLC separations of residue oil extracts obtained by Soxhlet (A) and pressurized liquid extraction (B) using *n*-hexane as extraction solvent on a Hypersil silica column. Chromatographic conditions: mobile phase, *n*-hexane:1,4-dioxane (96.0:4.0 v/v); flow rate, 1 ml min⁻¹; temperature, 40 °C. Peaks: (I) α -tocopherol, (II) α -tocotrienol, (IV) γ -tocotrienol, (V) δ -tocotrienol and (III and VI) unknown.

3.3. Comparison of Soxhlet extraction with pressurized liquid extraction

By using the optimized method obtained, a study of the repeatability of PLE was carried out. The contents of residue oil obtained from PPF were analyzed and compared with those obtained using Soxhlet extraction. Fig. 5 shows the average values and their corresponding standard deviations for the two extraction methods studied. The overall results

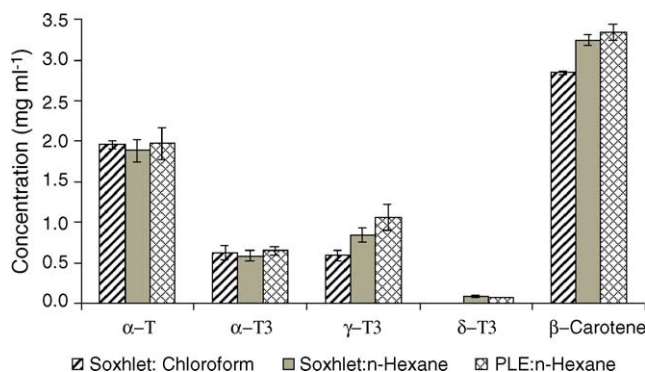


Fig. 5. Comparison of Vitamin E isomers and β -carotene concentration of optimized PLE and Soxhlet extraction (error bars represent standard deviation of results, $n = 4$).

Table 1

Comparison of results obtained on the determination of residue oil contents using various extraction methods

Parameter	Extraction method		
	Soxhlet (<i>n</i> -hexane)	Soxhlet (chloroform)	Pressurized liquid extraction (PLE)
Total extraction time (h)	8	8	25 min
Total solvent usage (ml)	~200	~200	~45
Total Vitamin E contents (mg ml ⁻¹)	3.1–3.5	3.3–3.6	3.7–4.0
Total β -carotene contents (mg ml ⁻¹)	3.1–3.3	2.8–3.0	3.3–3.5
Method repeatability (R.S.D., %) (<i>n</i> = 4)	3.0–6.0	3.0–6.0	<3.0

demonstrated that PLE method developed in this study is comparable with the Soxhlet extraction. In addition, by using same batch of sample, higher concentrations of γ -tocotrienol and α -tocotrienol in residue oil were obtained under PLE optimized conditions compared to those obtained by using the Soxhlet extraction. Based on the *t*-test results, no significant differences of the concentration of α -tocopherol and δ -tocotrienol were observed using various extraction methods in our study. The total quantity of carotene contents in the residue oil was also determined using the PORIM standard procedure. Based on the results illustrated in Fig. 5, the highest value of β -carotene was obtained by using *n*-hexane as solvent in PLE system. Soxhlet extraction generally resulted in lower values of β -carotene content and the values using *n*-hexane as extraction solvent were slightly higher than those using chloroform as extraction solvent. The overall RSD percent for each Vitamin E isomers detected and β -carotene concentrations using PLE system were less than 15%. Table 1 lists some of the extraction conditions and results for the three extraction methods used in this study. The results demonstrate that PLE gives total β -carotene and vitamin contents comparable to the Soxhlet extraction methods but offers the advantages of lower total organic solvent requirement and rapid extraction.

4. Conclusions

A novel and rapid method for the determination of Vitamin E isomers in PPF has been developed using home-made PLE extraction system and NPLC. The detailed comparison of Soxhlet with PLE showed the acceptable efficiency of PLE in terms of total extraction time, total solvent usage, total Vitamin E isomers contents as well as the method repeatability. PLE method demonstrated an outstanding performance with a total of four-fold reduction in total organic solvent consumption and up to 16-fold reduction in the total extraction time required against the Soxhlet extraction without significantly loss in extraction efficiency. Although the relative residue oil recovery for optimized PLE system (99.2%) is slightly lower than that for Soxhlet extraction (nominal value of 100%), slightly higher concentration of Vitamin E

isomers and β -carotene contents were observed. This could probably be due to the shorter extraction time afforded by PLE that consequently reduced the analytes degradation. Current work provides a great interest to further investigate on the applicability of the PLE method to the analysis of other minor components in the residue oil obtained from PPF such as carotenoids, sterols, phospholipids, and glycolipids.

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